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(54) Title: PROPHYLAXIS AND THERAPY OF ACQUIRED IMMUNODEFICIENCY SYNDROME			
(57) Abstract			
<p>The present invention involves a process for inducing resistance of an individual to infection by human immunodeficiency virus. The process involves vaccinating said individual with a synthetic peptide of mixture of peptides. The synthetic peptide(s) comprises an amino acid sequence derived at least in part from human immunodeficiency virus envelope protein conserved region. Upon antigenic presentation to an animal, this peptide induces directed cell-mediated immunity (i.e., T-cell cytotoxicity) to a substantially greater extent than production of antibody directed against native human immunodeficiency virus is elicited. The vaccine of the present invention comprises a synthetic peptide having an amino acid sequence derived at least in part from T-cell epitopes of human immunodeficiency virus envelope protein conserved region and preferably consists exclusively of T-cell epitopes.</p>			

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PROPHYLAXIS AND THERAPY OF
ACQUIRED IMMUNODEFICIENCY SYNDROME

15 The present invention concerns a method to prevent or
treat acquired immunodeficiency syndrome (AIDS) and
involves a new and novel approach for making a vaccine.
The vaccine comprises synthetic peptides which exhibit
certain immunological characteristics of one or more
20 proteins encoded by the viral causative agent of this
disease.

AIDS was first recognized in the United States in
1981; the number of cases has been increasing at a
25 dramatic pace since then. Since 1978 more than 2.4
million AIDS infections have been reported in the United
States, alone (Rees, Nature, 326:343, 1987). Once
significant immunosuppressive symptoms appear in an
infected individual, the expected outcome of the infection
30 is death. There is currently no known treatment that can
indefinitely delay or prevent the fatal consequences of
the disease. Although the disease first manifested itself
in homosexual or bisexual males and intravenous drug
abusers, it has now spread to others by means such as
35 intimate sexual contact with or receipt of blood products
from a carrier of the virus.

The causative agent, associated with AIDS has been identified as a group of closely related retroviruses commonly known as Human T Cell Lymphotropic Virus-type III (HTLV-III), Lymphadenopathy Viruses (LAV), AIDS-
5 Related Viruses (ARV), or more recently named Human Immunodeficiency Virus (HIV). These viruses will be collectively referred to herein for convenience as HIV.

Like other retroviruses, HIV has RNA as its genetic
10 material. When the virus enters the host cell, a viral enzyme known as reverse transcriptase copies the viral RNA into a double stranded DNA. The viral DNA migrates to the nucleus of the cell where it serves as a template for additional copies of viral RNA which can then be assembled
15 into new viral particles. The viral RNA can also serve as messenger RNA for certain viral proteins [either the viral core proteins (known as p18, p24 and p13)] or the reverse transcriptase, or be "spliced" into specific viral messenger RNAs necessary to produce several other viral
20 proteins including two glycosylated structural proteins known as gp41 and gp120 which are inserted in the outer membrane of the virus (Wain-Hobson et al., Cell 40:9, 1985). A recent study has shown that purified gp120 induces antibody in the goat, horse and rhesus monkey that
25 neutralizes HIV in lab tests (Robey et al., Proc. Natl. Acad. Sci., USA 83:7023, 1986).

Vaccines have been used for many years to prevent infections caused by agents such as viruses. The general
30 approach has been to inject healthy individuals with, for example, a killed or modified virus preparation in order to prime the individual's immune systems to mount an assault on the infecting virus. Recent advances in recombinant DNA technology have allowed safer methods of
35 vaccination that involve use of exposed viral components produced by microbial systems. After sufficient

purification, the viral component, for example a protein subunit, is administered as a vaccine in a suitable vehicle and/or an adjuvant. The latter stimulates the host's system in a way that improves the immune response to the viral subunit.

Another potential method of making a vaccine is by using chemically synthesized peptide fragments of a viral protein subunit. This method has several advantages over the other methods of producing vaccines, including purity of the product, reproducibility and specificity of the immune response.

Surface antigens of an infecting virus may elicit T cell and B cell responses. From the work of Milich and coworkers (Milich *et al.*, J. Exp. Med. 164:532, 1986; Milich and McLachlan, Science, 234:1398, 1986) it is clear that some regions of a protein's peptide chain may possess either T cell or B cell epitopes. These epitopes are frequently distinct from each other and may comprise different peptide sequences. Other examples include the work of Maizel *et al.*, (Eur. J. Immunol. 10:509, 1980) for hen egg-white lysozyme, and Senyk *et al.*, (J. Exp. Med., 133:1294, 1971) for glucagon. Thus, short stretches of a protein sequence (e.g. 15 amino acids) may elicit a T cell response but not a B cell response. A more complete review of these and other observations pertinent to this point is included in the work of Livingstone and Fathman (Ann. Rev. Immunol., 5:477, 1987).

A short peptide region within the surface protein of infectious Hepatitis B virus has been shown to elicit only a T cell response in mice (Milich *et al.*, 1986). Specifically, a synthetic peptide, whose sequence is derived from amino acids numbered 120-132 located within the pre-S(2) domain of the Hepatitis B surface antigen

gene, elicited a very strong T cell priming response to the peptide but stimulated only a very weak antibody response. In other words, mice mounted a poor antibody response to that peptide, but the T cells of immunized mice were efficiently primed (i.e. activated) to recognize that peptide as measured in T cell proliferation assays (Milich et al., 1986). The low level of the antibody produced by mice immunized with this peptide did not bind to the native viral surface antigen. The sequence of this T cell active peptide is:

Amino terminal-MQWNSTTFHQTLLQ-carboxy terminal.

The single letter code for amino acids used throughout this application is: A, alanine; C cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine.

In contrast to the above-described results, a second peptide sequence (amino acids 132-145) elicited a very weak T-cell response in mice (Milich et al., 1986). This second peptide did, however, efficiently bind antibody raised against it under conditions where a T cell epitope is provided.

The sequence of the second or B cell active peptide is:

Amino terminal-DPRVRGLYFPAGG-carboxy terminal.

Mice were also immunized with a longer peptide made up of both of the above-mentioned T- and B-active peptide sequences. In this case high titers of antibody were

produced against the B site peptide but not the T site peptide. The combination of both T- and B-sites within one peptide should stimulate both T and B cell responses, as measured by producing a specific antibody to the B cell epitope of the peptide chain. Synthetic peptide antigens may be constructed to produce two types of immune responses: T-cell only and T cell combined with a B cell response.

Cellular immune responses provide a major mechanism for reducing the growth of virus-infected cells (Doherty et al., Adv. Cancer Res., 42:1, 1985). A report by Earl et al., (Science, 234:728, 1986) demonstrated T-lymphocyte priming and protection against the Friend virus (a retrovirus)-induced mouse leukemia by a viral surface protein vaccine. Direct evidence for the role of a subset of T-lymphocytes (OKT8/LEU2 positive) in suppressing HIV growth in vitro was recently obtained by Walker et al., (Science, 234:1563, 1986). This study further demonstrated that, after depletion of CD8+ T-lymphocytes from the blood of HIV-infected individuals, large quantities of HIV were isolated from peripheral blood mononuclear cells of four of seven asymptomatic, seropositive homosexual men who were initially virus-negative or had very low levels of virus. Thus, the CD8+ subset of T-lymphocytes may play a role in virus infected individuals to prevent HIV replication and disease progression.

The present invention involves a process for inducing resistance of an individual to infection by human immunodeficiency virus. The process involves vaccinating said individual with a synthetic peptide or mixtures of synthetic peptides. The synthetic peptide(s) comprises an amino acid sequence derived at least in part from human immunodeficiency virus envelope protein conserved region.

Upon antigenic presentation, such a peptide induces directed cell-mediated immunity (i.e. T-cell cytotoxicity) to a substantially greater extent than production of antibody directed against native human immunodeficiency virus is elicited. The vaccine of the present invention comprises a synthetic peptide(s) having an amino acid sequence derived at least in part from T-cell epitopes of human immunodeficiency virus envelope protein conserved region and preferably consists exclusively of T cell epitopes.

The invention further predicts that the chemical nature and properties of the HIV surface proteins are similar to or may resemble proteins products of the immunoglobulin gene family in one or more biological characteristics. This group of genes includes the various immunoglobulins, the T cell receptor protein involved in antigen recognition, the major histocompatibility genes, the T4 antigen and others. This similarity will likely render HIV resistant to vaccines that induce an antibody response.

Just as HIV infects certain lymphoid cells, viruses like Human T Cell Leukemia virus type 1 (HTLV-1) and Feline Leukemia virus (FeLV) also infect and alter lymphocytes. HTLV-1 is associated with a T cell malignancy known as Adult T cell Leukemia (Yoshida and Seiki, Ann. Rev. Immunol 5:541, 1987). It is likely then that the surface proteins of both of these viruses also share one or more biological properties with the protein products of the immunoglobulin gene family and therefore will be resistant to vaccines that depend on antibody-induced inactivation of the infectious virus.

In greater detail, the process of the present invention for inducing resistance to human

immunodeficiency virus comprises several steps. Amino acid sequences of human immunodeficiency virus envelope protein conserved region able to form helical structures and further characterized by the presence of

5 amphipathically interrelated amino acids are first identified. Peptides or peptide derivatives comprising at least a substantial part of the identified sequences are then synthetically prepared. Said peptides or peptide derivatives are then administered to a test animal in a
10 manner stimulating an immune response. The T cell response and humoral antibody response in said test animal are monitored to screen for peptides or peptide derivatives which stimulate T-cell immunity without inducing substantial production of humoral antibody
15 directed against native human immunodeficiency virus. An individual is then inoculated with an immunogenic composition comprising said screened peptide or peptide derivative to induce resistance to human immunodeficiency virus infection.

20

The peptides or peptide derivatives of the present invention useful in prophylaxis of AIDS preferably comprise an amino acid sequence of human immunodeficiency virus envelope glycoproteins' conserved regions. The
25 human immunodeficiency virus envelope glycoproteins includes human immunodeficiency virus glycoprotein gp 120 and human immunodeficiency virus glycoprotein gp 41.

In situations where the treatment of individuals
30 already infected with HIV is desired, a T cell mediated immunity toward HIV-infected cells is also warranted. Such HIV-infected cells may express, on their surface, T cell epitopes of HIV envelope proteins and/or HIV core proteins. Thus, for such treatment, an immunizing peptide
35 or peptide derivative may have an amino acid sequence substantially comprising one or more T cell epitopes of a

HIV envelope protein or HIV core protein. The synthetic peptides of the present invention may be prepared by techniques involving solid-phase chemical synthesis, liquid-phase chemical synthesis or biological synthesis involving recombinant DNA, all well-known to those skilled in the relevant arts.

The HIV agent is unique in that it infects cells involved in the immune response and can kill these cells. The host cell often involved is the T4 lymphocyte, a white blood cell that plays a central role in regulating the immune system. The virus binds to cell surface T4 protein which is implicated in the mediation of efficient T cell-target cell interactions. T4+ lymphocytes interact with target cells expressing major histocompatibility (MHC) class II gene products. Both T4 and MHC genes are members of the immunoglobulin gene family (Maddon et al., Cell, 47:333, 1986). The observation that T4 interacts with the exterior HIV envelope protein, gp120, prompted a structural comparison of the viral protein to immunoglobulin proteins. Interestingly, two regions of gp120 were found to share sequence homology with human immunoglobulin heavy chain constant regions (Maddon et al., Cell, 47:333, 1986). Extrapolating from these observations, the present invention may hinge upon the fact that gp120 has certain properties unique to human immunoglobulins. Furthermore, this similarity in structure may allow the virus to escape inactivation by antibody interaction. Furthermore, viral-antibody interaction may, in certain situations, increase the infectivity of the virus. Recent work suggests that AIDS patients can and do have antibodies that neutralize the virus, as determined by in vitro lab tests. Yet these same patients die of the disease. The present invention predicts that antibodies binding to the virus may not interfere with and in some cases may even increase the

virus' inherent ability to infect the patient's lymphoid cells. Recently retrovirus infectivity was shown to be increased by binding of anti-retrovirus antibodies (Legrain et al., J. Virol., 60:1141, 1986). Therefore, an
5 AIDS vaccine that primes the individual's immune system to make antibodies to viral surface proteins may enhance the infectivity of an already deadly virus. What is needed then is to stimulate only the individual's T cell immunity (for example, cytotoxic T cells or CD8+ T cells) without
10 involving an antibody response to viral proteins. Synthetic peptide immunogens can certainly achieve this result.

The vaccine of the present invention is preferably a
15 totally synthetic vaccine made using a synthetic peptide(s) linked to a fatty acid compound, or polymerized through natural or extra cysteine residues. Important facets or considerations may be listed as follows for a vaccine of the present invention.

20

The vaccine of the present invention comprises short synthetic peptides. These short synthetic peptides (10-30 amino acids in length) have sequences from one or more conserved regions of either of the two HIV envelope.
25 These peptides should elicit a T cell response but not a substantial antibody response. Therefore, when suitably prepared, the peptide vaccine of the present invention will stimulate T cell immunity (i.e., cytotoxic T cells) without producing a substantial humoral antibody response.
30 The peptide-vaccine of the present invention should prime T cells in a way that, when the infecting virus appears at a later date, memory T cells will be activated to result in a cell-mediated immune response that will destroy the virus. The activation of only T cells without an antibody
35 response is important because it is believed that antibodies to most regions of the viral envelope protein

may stimulate the infectivity of the virus. This latter point will render most viral surface envelope antigen preparations (e.g., intact gp120 and gp41 that contain both B- and T-cell epitopes) ineffective as vaccines (see 5 article by D. Barnes in Science, 236:255, 1987). This article reported that about 20 chimpanzees had been given various prototype vaccines (containing B- and T-cell epitopes) and some were challenged by injecting virus, but the results indicated that none of the vaccines prevented 10 infection by infectious HIV). In contrast, this invention predicts that a suitable T cell response will produce cytotoxic T cells or other types of T-cell responses that will neutralize the virus in a newly infected individual.

15 It should be emphasized that an effective peptide may in some cases induce a low to moderate level antibody response and still be useful as an effective vaccine. In this case, the induced anti-peptide antibodies must be incapable of recognizing or detecting the mature protein 20 from which the vaccinating peptide was derived. Thus, the anti-peptide antibody induced by the T cell active peptide must not be substantially capable of binding to the intact, infectious virus. It is well known that anti-peptide antibodies to certain regions of a given protein 25 may not recognize the native protein (for example, see the work of Ho et al., J. Virol., 61:2024, 1987).

The use of synthetic peptides that are T cell-active but that are not immunogenic for native virus (anti-peptide antibodies that are unable to detect the virus 30 particle) may have some advantages in that inherent immunological memory should be superior for peptide vaccines of the present invention.

35 The first step in preparation of the vaccine of the present invention is to prepare a number of peptides 10-30

amino acids in length and having an amino acid sequence derived from the two envelope proteins or their genes. Conserved protein sequence regions of each envelope protein will be selected for investigation. For example,
5 a large portion of gp41 is conserved among the seven strains of HIV-sequenced to date (Modrow et al., J. Virol., 61:570, 1987).

Computer programs have been developed that are useful
10 in predicting T cell recognition sites and antibody binding sites within antigens (the latter known as B cell sites). Several computer programs can be used such as the De Lisi and Berzofsky program for T cell sites (Proc. Natl. Acad. Sci. USA, 82:7048, 1985), and for B cells- the
15 Hopp and Woods program (J. Mol. Biol., 157:105, 1982) and the Sette et al., program (Mol. Immunol., 23:807, 1986). Short synthetic peptides are made from predicted T cell regions.

20 Using the computer program of Sette et al., (1986) to analyze the linear sequence of the HIV envelope proteins, several proposed T cell epitopes were selected from a first conserved segment of gp120 (Modrow et al., J. Virol., 61:570-578). Their sequences are as follows, with
25 the amino terminus at the left and carboxy terminus on the right, in standard fashion:

- (1) CSAVEQLWVTVY;
- 30 (2) TTLFCASDAKAY;
- (3) EVVLGNVTENFNM;

(4) QMHEDIISLWDQS; and

(5) QSLKPCVKLTPLC.

5 These peptides are predicted T cell epitopes within a
100 amino acid stretch of conserved sequences near the
amino terminus of the gp120 protein. A recent report
indicated that this region is active in stimulating T cell
immunity (Ahearne et al., III International Conference on
10 AIDS, held in Washington, D.C., June 1-5, 1987, abstract
M.10.3, page 8).

Antigenic sites recognized by T cells have been
reported to correlate with helical structures (either
15 alpha helices or another type helix called a 3_{10} helical
structure). Such antigenic sites are also thought to be
protein segments displaying a polar/apolar character,
forming a stable amphipathic structure with separated
hydrophobic and hydrophilic surfaces and/or protein
20 segments displaying a marked change in hydrophilicity
between the first-half and the second-half of a block of
amino acids (differential amphipathic structures).

In practice, using computer programs, the helical
25 structures are identified by a consistent stretch of
blocks of amino acids (each block being 6-7 residues in
length) with angles (termed delta values) of $100^\circ \pm 20^\circ$
(alpha helix) or $120^\circ \pm 15^\circ$ (3_{10} helical structure).
Differential amphipathic structures are identified by
30 peaks of differential hydrophilicity (See Table 1). For
the purpose of selecting regions that are predicted to be
poor antibody eliciting and/or binding sites, these
structures should have negative mean hydrophilicity
values. All of these values are listed below in Table 1
35 as the computer analysis of a conserved gp120 sequence
(residues 35-137).

TABLE 1 con't

7	LWTVY	-1.69	4.3	80 145	3.40803117 6.79282004	7	LWTVYV	-1.15	5.5	88 180	5.78042364 12.6571412
8	WTVYV	-1.77	7.6	117 180	5.2587283 5.9999683	8	WTVYVYG	-1.55	5.6	80 180	3.90743985 10.9428538
9	VTVYVG	-1.61	2.8	80 180	2.27261392 .999999507	9	VTVYVGV	-1.28	5.2	80 112 180	5.68423569 7.47691013 12.4285601
10	TVYVGV	-1.61	1.2	80 180	2.36217496 .999999844	10	TVYVGVV	-1.45	0	100 180	10.0862161 11.5428464
11	VYVGVV	-1.54	3	80 144	1.85508868 1.91242888	11	VYVGVVV	-1.15	5.2	111 180	9.23573655 13.4571299
12	YVGVVV	-1.54	3.2	80 144	1.8054094 1.85463866	12	YVGVVVV	-1.42	4.7	80 180	2.88605369 11.7142808
13	YGVVVV	-1.59	7.3	113 180	4.82991766 7.09999547	13	YGVVVVV	-1.05	1.1	80 132	4.28917765 11.3842845
14	GVPVVK	.29	8	80 122	3.46214163 5.65042609	14	GVPVVKV	.27	10.5	80 118 180	5.05560131 7.81868378 10.6285662
15	VPVVKV	1.06	12.4	80 180	4.21156022 6.39999629	15	VPVVKVA	-1.05	12.4	80 112 180	6.62653296 9.64156172 12.2571317
16	PVVKVA	1.23	3.6	80 180	7.35037792 5.39999818	16	PVVKVAT	.65	4.1	87 180	11.1679208 8.14285043

TABLE 1 cont'd

17	VMKEAT	1.16	2.8	80 166	7.51038801 5.0399882	17	VMKEATT	.52	1	83 180	11.107197 7.02856624
18	WKEATT	1.35	10.7	80 120 180	3.91623012 3.30033045 3.89999705	18	WKEATTT	1.22	6.2	108	6.03090101
19	KEATTT	.71	6.7	80 115	3.87038548 3.54324115	19	KEATTTL	.55	8	94 166	10.8301427 6.61371042
20	EATTTL	-.09	4.7	108 180	4.84178659 4.89999533	20	EATTTLF	-.4	8.3	95 180	8.81271666 7.39999914
21	ATTTLF	-1	3.4	80 112 180	2.02960213 1.81597681 .599999107	21	ATTTLFC	-1.26	8.7	80 180	5.55101217 4.74285394
22	TTTLFC	-1.09	4.1	80 136	2.54250479 1.39839332	22	TTTLFCA	-1.26	9.2	80 180	4.52553122 3.65714207
23	TTLFCA	-1.11	1.4	80 157	3.3632097 .332518758	23	TTTLFCAS	-1.25	1.1	80 130	5.52825776 5.40023235
24	TLFCAS	-.99	3.5	80 147	3.32553457 1.80392438	24	TLFCASD	-.85	8.4	80 119 180	3.87846076 7.0806631 5.94284743
25	LPCASD	-.42	8.1	80 122 180	2.06205252 4.25830797 2.49999907	25	LFCASDA	-1.21	11.6	80 115 180	4.08829296 6.42577424 4.99999328
26	FCASDA	-.21	6.8	80 139	3.58038047 4.84654054	26	FCASDAK	-.11	12.7	94 150	5.27711073 9.17980169

-15-

TABLE 1 cont'd

27	CASDAK	.71	6.7	113 180	3.91223995 6.69999644	27	CASDAKA	.04	3.8	80 123 180	3.77241193 7.36122595 10.9428509
28	ASDAKA	.8	.8	80 157	2.28563281 6.39032513	28	ASDAKAY	.58	.9	80 150	3.54848783 10.4603253
29	SDAKAY	.5	2.6	80 138	3.2211804 6.65130434	29	SDAKAYS	.95	2.2	80 180	.944789442 11.6571397
30	DAKAYS	.5	8	80 136	3.01974827 6.39531508	30	DAKAYST	.94	2.8	80 180	.97233936 11.2571407
31	AKAYST	-.07	4.4	115	5.43528965	31	AKAYSTE	.94	4.7	81 148	4.07367275 10.3211839
32	KAYSTE	.51	2.7	80 144	6.27726053 6.09214551	32	KAYSTEY	.6	3.4	81 146	8.27920693 13.1855076
33	AYSTFV	-.24	3.6	80 180	4.62499407 6.99999752	33	AYSTEYH	-.03	1.49	80 139	5.29178076 9.29903238
34	YSTEYH	.51	7.9	105 180	5.1518362 11.4999928	34	YSTEYHN	.72	4	117	9.71864323
35	STEYHN	.93	.2	80 180	.963303906 8.99999872	35	STEYHNV	-.06	6.2	128	12.8286227
36	TEYHNV	.63	1.6	80 146	3.30736594 8.1502864	36	TEYHNVN	-.05	.19	127	13.7231702
37	EVHNVN	1.26	3.4	134	10.0166439	37	EVHNVNA	-.4	3.9	138	14.2893846

TABLE 1 con't

38	VHNVNA	.68	1.3	131	9.91647749	38	VHNVWAT	-.81	1	94 148	8.01966306 10.1310171
39	HNVNAT	.86	.19	116	8.66449943	39	HNVNATH	-.28	.4	88 180	4.30849285 11.8285674
40	NVWATH	.86	1	123	8.65369771	40	NVWATHA	-.46	1.8	84 161	5.89730511 10.5891462
41	VWATHA	.75	1.69	133	9.64004539	41	VWATHAC	-1.32	.29	80 133 180	2.72499528 4.94525044 7.48570996
42	NATHAC	.83	0	112	8.84659018	42	NATHACV	-1.32	8.3	87 149	3.62934111 5.91098659
43	ATHACV	.01	6.1	80 180	6.1106703 4.899999	43	ATHACVP	-1.22	3.5	80 146	6.638677 6.75061693
44	THACVP	.09	5.6	80 180	5.55283546 5.39999851	44	THACVPT	-.86	.29	80 135	6.79346425 5.22522487
45	HACVPT	.1	4.4	80 180	5.17273346 5.39999715	45	HACVPTD	-.46	10.6	80 180	5.70807306 7.8571391
46	ACVPTD	-.07	5.6	80 180	2.90366341 4.39999876	46	ACVPTDP	-.16	14.3	80 180	3.93828552 6.14285612
47	CVPTDP	.01	5.1	80 158	3.3008536 3.98700801	47	CVPTDPN	.59	13.7	98 180	6.06728507 7.3999986
48	VPTDPN	.21	5.1	80 180	3.85549613 5.09999822	48	VPTDPNP	1.18	8.6	102 180	5.52642539 10.0857082

TABLE 1, con't

49	PTDPNP	.46	2.4	88 180	3.38300557 3.59999964	49	PTDPNPQ	2.28	2.8	93 180	2.49422442 5.91428331
50	TDPNPQ	.5	2.2	80 170	2.71846268 3.80175964	50	TDPNPQE	2.55	2.8	91 180	2.95517847 5.65714194
51	DPNPQE	1.06	0	80 142	4.14976691 4.8530168	51	DPNPQEV	1.85	5.8	90 144	7.87118593 9.90899754
52	PNPQEV	.31	1.5	143	4.58159443	52	PNPQEVV	.75	11.6	104 180	9.82802 1.05713855
53	NPQEVV	.06	.4	100	4.89667866	53	NPQEVVL	-.02	20.8	80 147	6.77921655 6.79771978
54	PQEVVL	-.27	8	80 180	4.88563707 4.59999854	54	PQEVVLG	-.46	16.2	80 180	10.712972 6.25713895
55	QEVVLG	-.54	6.6	80 136	4.22460786 4.03179431	55	QEVVLGN	-.19	2.7	80 133	13.0618563 7.43411692
56	EVVLGN	-.54	3.2	80 143	4.7537475 5.23077801	56	EVVLGNV	-1.29	4.6	80 148	14.8577324 12.1496517
57	VVLGNV	-1.29	1.9	80 132	1.81938005 1.94924385	57	VVLGNVT	-1.69	12.2	80 146	7.97331492 9.18636992
58	VVLGNVT	-1.11	3.2	80 180	1.36359393 2.59999936	58	VVLGNVTE	-.59	7.6	98	14.3811965
59	LGNVTE	-.36	4.3	115 180	6.05158251 1.89999906	59	LGNVTEN	.51	7.6	99	13.327365

TABLE 1 cont'd

60	GNVTEN	-.02	5.7	80 130	3.07264158 5.08870785	60	GNVTENF	.65	4.5	96 180	12.4977417 11.4571327
61	NVTENP	-.17	2.4	95 180	6.95264042 .999997219	61	NVTENPN	1.1	4.2	104 180	13.410322 13.599856
62	VTENPN	-.17	3.2	94 180	6.87502696 .99999923	62	VTENPNM	.32	1.2	110 180	10.3891878 13.4285586
63	TENPNM	-.14	6.4	80 128	4.31543897 5.52726887	63	TENPNMW	1.05	5.2	80 145	3.03805257 9.71945235
64	ENPNMW	.5	1.6	80 146	6.56133261 7.0171153	64	ENPNMWK	1.51	1.3	80 134	2.85710325 11.1247084
65	NPNMWK	.5	7.2	80 147	4.83053688 5.17803036	65	NPNMWKN	1.51	4.1	80 180	3.36279834 10.6857106
66	FNMWKN	.5	10.2	80 147	5.34455775 5.54090488	66	FNMWKNN	1.51	12.1	148	9.50267953
67	NMWKNN	.94	1.09	84	6.47598203	67	NMWKNNM	1.64	2.6	80 147	10.3115109 8.85827898
68	MWKNNM	.7	6	80 140	6.10042863 5.32945602	68	MWKNNMV	.54	5.5	80 127 180	9.63372205 7.14594883 1.74284865
69	MWKNNMV	.66	9.2	80 120 180	2.50153618 4.27897212 .599996358	69	MWKNNMVE	1.31	10.9	80 141	11.0435161 7.54448264

TABLE 1 cont'd

70	KNNVZ	.6	3.2	80 142	5.58419524 6.14937436	70	KNNVZ	1.68	8.1	80	12.1178417
71	NNVZ	.13	2.6	96	4.73472565	71	NNVZ	.85	0	90	14.9537026
72	NNVZ	-.12	4.5	100	6.00883252	72	NNVZ	.28	3.7	96 180	14.1948524 2.31428043
73	MVEQMH	.51	2.7	115	9.19182903	73	MVEQMH	.28	3.7	97 180	14.1805622 4.1142807
74	VEQMH	1.23	4	116	8.62558323	74	VEQMH	1.05	3.7	94 149	14.283656 5.84410789
75	EQMH	1.98	8.1	80	6.34436198	75	EQMH	1.39	.1	91 180	12.326519 4.59999931
76	QMH	.31	3.9	80 180	12.6132845 12.49999944	76	QMH	.64	1.2	90 166	12.1656749 4.38843143
77	MH	-.89	16.7	80 147	14.797617 7.21621384	77	MH	.25	3.9	80 157	10.5667494 1.58692131
78	HED	-.62	23.7	80 141	15.0471601 7.85709809	78	HED	-.02	11.3	114 180	9.62047164 5.08570813
79	ED	-1.59	7.5	81 180	15.6576936 2.09999728	79	ED	.18	7.3	118 180	9.13994576 5.31428206
80	DI	-1.52	12.9	80 140	12.6258975 13.6872029	80	DI	.18	.69	80 123 180	2.75431712 11.6897921 1.31428269

TABLE 1 (con't)

81	IISLWD	-1.52	18.3	80 124	4.8601113 10.62247	81	IISLWDO	.18	10.7	100 180	7.92396483 5.31428532
82	ISLWDO	-.32	15.1	80 164	6.41076427 9.73681878	82	ISLWDOQS	.55	12.6	80 180	6.14412544 7.05713941
83	SLWDQS	.9	1.6	87	6.61515331	83	SLWDQSL	.27	2.6	80 147	12.1215222 7.59794494
84	LWDQSL	.55	5.9	80 141	6.51625779 6.2593098	84	LWDQSLK	.71	.3	81 149	13.5587838 10.5987673
85	WDQSLK	1.35	5.1	80 151	5.92780332 5.29558905	85	WDQSLKP	1.48	6.2	89 180	9.49759941 7.48571071
86	DQSLKP	.78	2.3	87 180	5.08496961 7.89999554	86	DQSLKPC	1	4.8	97	12.4768136
87	QSLKPC	.11	3.3	122	5.20035816	87	QSLKPCV	-.1	5.6	95 159	14.484075 5.67278754
88	SLKPCV	-.17	4	89 180	4.72437198 5.5999792	88	SLKPCVK	-.05	3.7	100 180	14.2863777 12.542844
89	LKPCVK	.28	.7	97 180	7.84178318 8.29999305	89	LKPCVKL	-.7	5.8	107 180	14.9608929 14.799984
90	KPCVKL	.28	2.3	99 180	7.08316366 8.29999385	90	KPCVKLT	-.06	2.2	107 180	11.1829906 12.4571346
91	PCVKLT	-.29	3.3	132	5.51747666	91	PCVKLTP	-.39	3.6	127	12.9245369

-22-

TABLE 1 con't

92	CVKLTTP	-1.29	2.7	129	5.47302639	92	CVKLTPL	-1.16	1.3	80 133	-824544342 13.7445971
93	VKLTPPL	-1.42	1.9	80 138	1.72145914 6.86168129	93	VKLTPPLC	-1.16	.6	80 132	2.17205276 12.8299583
94	KLTPLC	-1.34	3.6	124	5.85689593						

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Five peptides were selected from within residues 35 through 137 of the gp120 surface protein of HIV.

Peptide number (1) which spans blocks 1-5 (6 amino acids per block) has delta values (termed ANGLE) consistent with a helical structure as predicted by both the Hopp/Woods computer program (block length of 6 amino acids) and the Kyte/Doolittle computer program (block length of 7 amino acids).

10

Peptide number (2) which spans blocks 23-28 has a peak of differential hydrophilicity (a marked change in mean hydrophilicity between the first-half and second-half of a block of amino acids) that is predicted by both programs.

15

Peptide number (3) which spans blocks 56-63 has delta values consistent with a helical structure (Kyte/Doolittle) and a peak of hydrophilicity (both programs).

20

Peptide number (4) which spans blocks 76-83 has a peak of differential hydrophilicity (both programs).

Peptide number (5) which spans blocks 87-94 has delta values consistent with helical structures (both programs).

25

All five of these peptides exhibit negative mean hydrophilicity values indicating that they are poor antibody binding sites.

30

Five other conserved regions of the two HIV envelope proteins can be similarly analyzed and putative T cell-active peptides selected. These regions include residues 204-279 (C2 or conserved region 2), 415-458 (C3), 470-510

35

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(C4), 511-616 (C5) and 654-745 (C6) (Modrow et al., J. Virology, 61:570,1987).

As an alternate approach to identify T cell active peptides, it may be necessary to thoroughly cover the protein sequence in question. In this case, overlapping 15-amino acid peptides (15 mers) can be made (the second peptide overlaps with the C-terminal 5 amino acids of the first peptide, the third overlaps the second, etc.) across the complete conserved amino acid sequence of both gp120 and gp41.

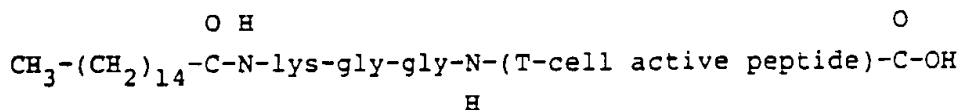
All of these peptides may be made, for example, by the solid phase Merrifield-type synthesis but may also be made by liquid phase synthesis or recombinant DNA-related methods known to those skilled in the relevant arts. A further description of the basic solid phase synthesis method, for example, can be found in the literature (i.e., M. Bodansky et al., Peptide Synthesis, John Wiley and Sons, Second Edition, 1976, as well as in other reference works known to those skilled in this type of chemistry. Appropriate protective groups usable in such synthesis and their abbreviations will be found in the above reference, as well as in J.F.W. McOmie, Protective Groups in Organic Chemistry, Plenum Press, New York, 1973).

In one type of synthesis, the N-terminal end of each peptide is linked to a dipalmityl-lysyl-glycyl-glycyl sequence to serve as a carrier as described by T.P. Hopp (Mol. Immunol., 21:13, 1984).

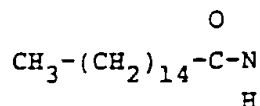
An example of this type of structure is shown below:

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= alpha amino group of lysine



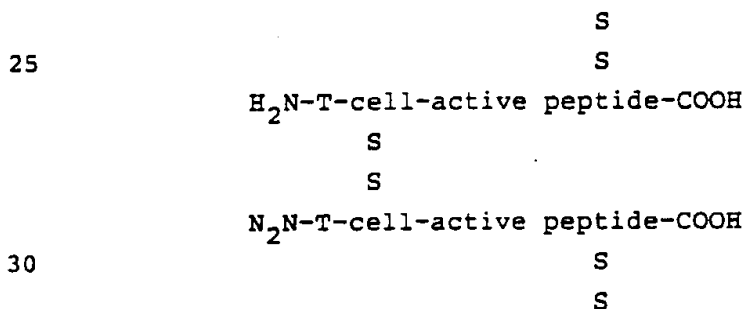
5



= epsilon amino group of lysine

- 10 Alternately, peptides can be made without the use of the dipalmitate carrier and otherwise tested. In this case, peptides containing two natural cysteines as part of their natural sequence may be selected and synthesized. Peptides lacking such cysteines can be modified by the
- 15 addition of extra cysteines to the N- and C-terminal ends, respectively. The presence of two cysteines per peptide allow polymerization of the subunit peptide by air oxidation to form cysteine-linked polymers and/or cyclic peptides. Such polymers should enhance immune recognition
- 20 of the peptide without the need of a carrier.

An example of this type of structure is shown below:



- Each peptide preparation will first be tested in mice, for example, to screen for appropriate T cell active
- 35 peptides. T cell active peptides will be assayed by injecting the peptide into mice, and then testing T cells

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- recovered from the murine lymph nodes one to three weeks after inoculation with the peptide. The measurement of activation or priming of T cells will be done by T cell proliferation tests and/or interleukin-2 production (Milich et al., J. Exp. Med., 164:532, 1986). Two types of T cell active peptides should be found. The more prevalent group of peptides will prime T cells that respond in test tube assays to only the peptide and not the corresponding native HIV surface protein. The second group of peptides will prime T cells to respond to both the peptide and the native HIV protein. It is this latter group of peptides that will induce protective immunity in the vaccinated host. Several strains of mice will be used which vary in their histocompatibility genes. Peptides that have a broad response in the various MHC genotypes will be selected for further study in primates, finally humans.

- T cell active peptides will then be screened to identify those peptides that lack B cell stimulatory activity. This will be accomplished by injecting each peptide into small animals (various strains of mice) to identify those peptides that fail to generate an antibody response. These animals should not produce anti-peptide antibodies binding to native viral proteins. These same selected peptides will be tested in baboons and monitored to confirm the lack of anti-peptide antibody production in baboon sera. At this stage, mixtures of peptides will be employed because it is quite possible that one peptide sequence will not provide the broad spectrum coverage needed for an effective vaccine. Candidate peptide mixtures will then be incorporated into a vaccine. Candidate peptide mixtures will then be tested in a suitable animal that allows replication of the AIDS virus (Chimpanzees) to test for priming of T cells. Peptides that are more active will be used to vaccinate chimpanzees

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in a virus challenge experiment. A successful protection experiment will prevent viremia without eliciting a significant humoral antibody response but will prime T cells for in vitro responses to the envelope antigens.

- 5 The virus will be neutralized by cell mediated immunity. The present invention involves the prediction that antibody responses to most if not all surface antigen epitopes will increase or at least not impede the infectivity of the AIDS virus.

10

- As described above, it may not be necessary to select a peptide that completely lacks the capability to raise anti-peptide antibodies. In this situation, the anti-peptide antibody must not be capable of recognizing the
15 native envelope proteins as measured, for example, either by immunoblotting procedures or by other immunoabsorbent (ELISA) tests. What is important in this particular response is that anti-peptide antibodies against a certain peptide sequence must not induce antibodies that bind to
20 the infectious virus. Thus, in this case, T cell active peptides that raise low or moderate levels of anti-peptide antibodies will be screened to identify those that fail to detect either intact virus preparations or viral surface proteins by immunoabsorbent tests (ELISA) and/or
25 immunoblot procedures.

- An important issue in considering the effectiveness of this invention is whether the cell mediated immune system can function in a previously vaccinated individual
30 when at a later time the vaccinee is exposed to HIV which is infecting and altering the function of T4 helper cells. The research findings of Buller et al. (Nature, 328: 77, 1987) provide evidence that is consistent with the hypothesis that a T cell active peptide can invoke a cell
35 mediated response in the absence of T4 helper cells. Their work demonstrates that cytotoxic T cell responses
-

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can be induced in mice in the absence of T helper cells; the end result was that mice being studied recovered from a viral disease without T helper cells.

5 Therapy for HIV-infected people is also an object of the present invention. Although the synthetic vaccine of the present invention will focus on peptides sequences predicted from one of the viral surface proteins in order to prevent virus infection of the exposed individual, this
10 approach might also be used to treat individuals who are already infected with HIV. In this particular situation, it is important to consider that the target for cell-mediated immunity includes not only the virus but more importantly the virus-infected cell. Infected cells will
15 have not only viral envelope proteins on their surfaces but possibly glycosylated core proteins (gag gene products) or their higher molecular weight precursors as well (Naso et al., J. Virol., 45:1200, 1983). Therefore, T cell active peptides from the gag gene of HIV can also
20 be selected and tested for their affects on virus infected cells.

Computer analysis of the gag gene of HIV has revealed several T cell epitopes from within the core or gag gene
25 of HIV (Coates et al., Nature, 326:549, 1987).

-29-

56 62
 EGCRQIL
 74 85
 ELRSLYNTVAT
 5 170 180
 VIPMFSALSEG
 199 206
 AMQMLKET
 298 305
 10 YVDREYKT
 333 342
 KTILKALGPA
 346 355
 EMMTACQGV
 15 367 375
 AEAMSQVTN

Such synthetic peptides (either from the surface proteins or the core proteins) should be able to induce a cell-mediated response sufficient to destroy virus-infected cells bearing the expected epitopes, or as suggested by the work Walker et al., (Science, 234:1563-1566, 1986) inhibit the growth of the virus.

The T helper cell independent cytotoxic T cell response, described by Buller et al., bodes well for the use of T cell active peptides in the therapy of AIDS. Such a peptide or mixture of peptides would be expected to mount an effective cell mediated immune response at a time when T4 cells are being infected and killed by the HIV. Since T8 cells are resistant to HIV infection, the proposed peptide(s) (either polymerized or coupled to fatty acids as described in a previous section) should activate and prime T8 cytotoxic cells allowing a specific virus-killing response in the AIDS patient even though the

- 30 -

virus may be infecting and altering the immune helper function of T4 cells.

Studies of Walker et al., (Nature, 328: 345, 1987) have demonstrated the presence HIV-specific cytotoxic T cells in persons infected with HIV. These cytotoxic T cells were able to kill HIV-antigen containing B lymphocytes derived from the same patient in laboratory tests. Their study showed that monoclonal antibody specific for cytotoxic T cells was able to inhibit the cell killing activity. These results support the vaccine approach described in this patent, and may have important implications for the use of T-cell active peptides in the treatment of AIDS patients.

15

* * * * *

Changes may be made in the construction, operation and arrangement of the various parts, elements, steps and procedures described herein without departing from the concept and scope of the invention as defined in the following claims.

20

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CLAIMS:

1. A process for inducing resistance of an individual to
5 infection by human immunodeficiency virus, the process
involving the steps of vaccinating said individual with a
synthetic peptide or mixture of peptides comprising a
sequence of from about 10 to about 30 amino acids derived
at least in part from human immunodeficiency virus
10 envelope protein conserved regions and which, upon
antigenic presentation to an animal, induces directed
cell-mediated immunity (including a T-cell cytotoxicity
response to AIDS virus) to a substantially greater extent
than it elicits production of antibody directed against
15 native human immunodeficiency virus.

2. A process for inducing resistance of an individual to
infection by human immunodeficiency virus, the process
20 involving the steps of treating said individual with a
vaccine consisting essentially of a synthetic peptide, or
mixture of peptides, having an amino acid sequence derived
from T-cell epitopes of human immunodeficiency virus
envelope protein conserved region.

25

3. A process for inducing resistance to human
immunodeficiency virus, the process involving the steps
of:
30 identifying amino acid sequences of about 10 to about
30 amino acids from human immunodeficiency virus
envelope protein conserved region able to form a
helical structure and being further characterized
by the presence of amphipathically interrelated
35 amino acids;

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preparing peptides or peptide derivatives comprising at least substantial parts of the identified sequences;

5 administering said peptide, or mixture of peptides, or peptide derivatives to a test animal in a manner stimulating an immune response;

10 monitoring T-cell activity and humoral antibody response in said test animal to screen for a peptide which stimulates T-cell activity without inducing substantial production of antibody directed against native human immunodeficiency virus to identify peptides exclusively having T
15 cell epitopes; and

treating an individual with an immunogenic composition comprising exclusively with T cell epitopes peptides to induce resistance to human
20 immunodeficiency virus infection.

4. A process for inducing resistance to human immunodeficiency virus, the process involving the steps
25 of:

preparing peptides substantially comprising the structure:

30 CSAVEQLWVTVY,
TTLFCASDAKAY,
EVVLGNVTENFNM,
QMHEDIISLWDQS, or
35 QSLKPCVKLTPLC;

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screening for a peptide, or mixture of peptides, which,
upon administration to an animal, stimulates T-
cell activity without induction of substantial
production of antibody directed against native
human immunodeficiency virus; and

administering said screened peptide to a human to
induce resistance to human immunodeficiency virus
infection.

5. The process of claim 4 wherein the peptide is
comprised in a human immunodeficiency virus envelope
glycoprotein.

6. The process of claim 4 wherein the peptide is
comprised in human immunodeficiency virus glycoprotein gp
120.

7. The process of claim 4 wherein the peptide is
comprised in human immunodeficiency virus glycoprotein gp
41.

8. A process for suppressing infection by human
immunodeficiency virus, the process involving the steps
of:

preparing a peptide, or mixture of peptides,
substantially comprising the sequence:

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EGCRQIL;
ELRSLYNTVAT;
VIPMFSALSEG;
AMQMLKET;
5 YVDREYKT;
KTILKALGPA;
EMMTACQGV; or
AEAMSQVTN;

10 screening for a peptide (or peptides) which, upon
administration to an animal, stimulates T-cell
activity without inducing substantial production
of antibody directed against native human
immunodeficiency virus; and

15 administering said screened peptide(s) to a human to
induce resistance to human immunodeficiency virus
infection.

20 9. The process of claim 8 wherein the peptide(s) is
comprised in a human immunodeficiency virus core protein
and T-cell cytotoxicity is directed toward cells infected
with human immunodeficiency virus.

25 10. A vaccine for the prevention of infection by human
immunodeficiency virus, the vaccine comprising a synthetic
peptide(s) having an amino acid sequence derived from
30 that of conserved regions of human immunodeficiency virus
envelope protein, said vaccine inducing a T-cell mediated
response against human immunodeficiency virus but not a
substantial production of humoral antibody against human
immunodeficiency virus.

35

- 35 -

11. The vaccine of claim 10 wherein the synthetic peptide(s) comprises a sequence of between about 6 and about 30 amino acids.

5

12. The vaccine of claim 10 wherein the synthetic peptide is prepared by solid-phase chemical synthesis, liquid-phase chemical synthesis or biological synthesis involving recombinant DNA.

10

13. A method for therapy of a human immunodeficiency virus-infected patient with AIDS, the method comprising treatment with a vaccine comprising a synthetic peptide(s) having an amino acid sequence derived from that of a human immunodeficiency virus protein, said synthetic peptide eliciting a T-cell response but not a substantial production of humoral antibody against native human immunodeficiency virus protein.

20

14. The method of claim 13 wherein the human immunodeficiency virus protein is a core protein.

25

15. The method of claim 13 wherein the human immunodeficiency virus protein is an envelope protein.



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(54) Title: PROPHYLAXIS AND THERAPY OF ACQUIRED IMMUNODEFICIENCY SYNDROME			
(57) Abstract			
<p>The present invention involves a process for inducing resistance of an individual to infection by human immunodeficiency virus. The process involves vaccinating said individual with a synthetic peptide of mixture of peptides. The synthetic peptide(s) comprises an amino acid sequence derived at least in part from human immunodeficiency virus envelope protein conserved region. Upon antigenic presentation to an animal, this peptide induces directed cell-mediated immunity (i.e., T-cell cytotoxicity) to a substantially greater extent than production of antibody directed against native human immunodeficiency virus is elicited. The vaccine of the present invention comprises a synthetic peptide having an amino acid sequence derived at least in part from T-cell epitopes of human immunodeficiency virus envelope protein conserved region and preferably consists exclusively of T-cell epitopes.</p>			

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INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 88/02970

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC IPC4: A 61 K 39/21, C 07 K 7706, 7708																	
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched †</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 30%; border-bottom: 1px solid black;">Classification System</th> <th style="width: 70%; border-bottom: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="border: 1px solid black; height: 40px; vertical-align: top; padding: 5px;">IPC4</td> <td style="border: 1px solid black; height: 40px; vertical-align: top; padding: 5px;">A 61 K</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ‡</div>			Classification System	Classification Symbols	IPC4	A 61 K											
Classification System	Classification Symbols																
IPC4	A 61 K																
III. DOCUMENTS CONSIDERED TO BE RELEVANT † <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; text-align: left;">Category *</th> <th style="width: 60%; text-align: left;">Citation of Document, †† with indication, where appropriate, of the relevant passages †‡</th> <th style="width: 30%; text-align: left;">Relevant to Claim No. †‡</th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top;">X</td> <td style="vertical-align: top;">Proc. Natl. Acad. Sci. USA, Vol. 84, June 1987 Kemp B. Cease et al.: "Helper T-cell antigenic site identification in the acquired immunodeficiency syndrome virus gp120 envelope protein and induction of immunity in mice to the native protein using a 16-residue synthetic peptide", page 4249-4253 see fig. 1, last 4 lines (envT2) and page 4252, right col. 3rd paragraph</td> <td style="vertical-align: top;">10-12</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">Y</td> <td style="text-align: center; vertical-align: top;">--</td> <td style="vertical-align: top;">10-12</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">X</td> <td style="vertical-align: top;">FEBS LETTERS, Vol. 218, No. 2, June 1987 M.J.E. Sternberg et al.: "Prediction of antigenic determinants and secondary structures of the major AIDS virus proteins ", see page 231 - page 237 see in particular fig. 3 page 236 "CONCLUSION"</td> <td style="vertical-align: top;">10-12</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">Y</td> <td style="text-align: center; vertical-align: top;">--</td> <td style="vertical-align: top;">10-12</td> </tr> </tbody> </table>			Category *	Citation of Document, †† with indication, where appropriate, of the relevant passages †‡	Relevant to Claim No. †‡	X	Proc. Natl. Acad. Sci. USA, Vol. 84, June 1987 Kemp B. Cease et al.: "Helper T-cell antigenic site identification in the acquired immunodeficiency syndrome virus gp120 envelope protein and induction of immunity in mice to the native protein using a 16-residue synthetic peptide", page 4249-4253 see fig. 1, last 4 lines (envT2) and page 4252, right col. 3rd paragraph	10-12	Y	--	10-12	X	FEBS LETTERS, Vol. 218, No. 2, June 1987 M.J.E. Sternberg et al.: "Prediction of antigenic determinants and secondary structures of the major AIDS virus proteins ", see page 231 - page 237 see in particular fig. 3 page 236 "CONCLUSION"	10-12	Y	--	10-12
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Y	--	10-12															
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: †§</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>																	
IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;"> Date of the Actual Completion of the International Search 29th March 1989 </td> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;"> Date of Mailing of this International Search Report 18. 04. 89 </td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px;"> International Searching Authority EUROPEAN PATENT OFFICE </td> <td style="border-bottom: 1px solid black; padding: 5px;"> Signature of Authorized Officer P.C.G. VAN DER PUTTEN </td> </tr> </table>			Date of the Actual Completion of the International Search 29th March 1989	Date of Mailing of this International Search Report 18. 04. 89	International Searching Authority EUROPEAN PATENT OFFICE	Signature of Authorized Officer P.C.G. VAN DER PUTTEN											
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CORRECTED VERSION

International Application No. PCT/US 88/02970

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	EP, A2, 0 203 676 (THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY) 3 December 1986, see page 6, lines 11-14 --	10-12
A	NATURE, Vol. 326, April 1987 A.R.M. Coates et al.: "AIDS Vaccine Predictions", see page 549 - page 550 see the whole document --	10-12
A	Cell, Vol. 45, June 1986 B.R. Starcich et al.: "Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS", see page 637 - page 648 see the whole document --	10-12
A	JOURNAL OF VIROLOGY, Vol. 61, No. 2, February 1987 Susanne Modrow et al.: "Computer-assisted analysis of envelope protein sequences of seven human immunodeficiency virus isolates: prediction of antigenic epitopes in conserved and variable regions", see page 570 - page 578 see the whole document --	10-12
P,X	EP, A2, 0 273 716 (THE UNITED STATES OF AMERICA) 6 July 1988, see in particular claims 2 and 9 and table 3 --	10-12
P,X	WO, A1, 88/05440 (INSTITUT PASTEUR) 28 July 1988, see claims 8-11 and fig. 2 --	10-12
P,A	EP, A2, 0 260 714 (ONCOGEN) 23 March 1988, see the whole document --	10-12
P,A	EP, A2, 0 279 994 (THE UNITED STATES OF AMERICA) 31 August 1988, see the whole document -- -----	10-12

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 1-9, 13-15 because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv)

Method for treatment of the human or animal body by means of surgery or therapy, as well as diagnostic methods.

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This international Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. PCT/US 88/02970**

SA 26132

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 12/01/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0 203 676	03/12/86	NONE	
EP-A2- 0 273 716	06/07/88	WO-A- 88/05051 AU-D- 13657/88 JP-T- 63503227	14/07/88 27/07/88 24/11/88
WO-A1- 88/05440	28/07/88	EP-A- 0283327 AU-D- 12250/88 FR-A- 2610632 FR-A- 2614025	21/09/88 10/08/88 12/08/88 21/10/88
EP-A2- 0 260 714	23/03/88	JP-A- 63119428	24/05/88
EP-A2- 0 279 994	31/08/88	WO-A- 88/04935 AU-D- 12277/88	14/07/88 27/07/88

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82